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Functional Significance of the Co-Localization of Taste Buds and Teeth in the Pharyngeal Jaws of the Largemouth Bass, *Micropterus salmoides*

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Abstract. Studies of feeding behavior in the largemouth bass, *Micropterus salmoides*, revealed that live goldfish or artificial food balls are ingested in three discrete steps: inhalation of the food into the oral cavity, passage through the pharyngeal cavity, and swallowing. Food balls with or without a feeding stimulant were inhaled with equal frequency; thus, vision was clearly the major sense affecting inhalation. However, food balls with defined concentrations of a feeding stimulant were swallowed in a dose-dependent manner, whereas food balls without a feeding stimulant were promptly expelled. Thus, gustation played a major role in stimulating swallowing. Videotaped observations of feeding behavior suggested that both food processing and gustation occur in the pharynx and take place before the swallowing of either goldfish or food balls. The well-developed pharyngeal jaws of largemouth bass consist of six major pads of caniform teeth in the upper pharynx and two pads in the lower pharynx. Scanning electron microscopy showed that taste buds were abundant around most of these pharyngeal teeth. Histological sections prepared from all pharyngeal pads revealed that both elevated and flattened taste buds occur with the teeth. The morphology of these taste buds was typical of that described in other teleosts. Neuronal profiles, visualized with an HNK-1 monoclonal antibody, were observed entering each taste bud. The antibody also selectively stained a group of one to four putative sensory cells in each taste bud and the distal processes of these cells in the receptor area. The co-localization of teeth and taste buds on the pharyngeal jaws indicates that food processing and gustation both occur there, and that together these processes determine whether a potential food item is swallowed.

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Introduction

Live fish are a major food item in the diet of the largemouth bass, *Micropterus salmoides* (Howick and O'Brien, 1983). Although this species is primarily a visual feeder (McMahon and Holanov, 1995), gustation apparently plays a role in the ultimate acceptance (Kubitza and Lovshin, 1997) or rejection (Kruse and Stone, 1984) of potential food items.

Taste cells present in multicellular taste buds are the major functional units in the gustatory sense of all vertebrates including teleosts (*e.g.*, Roper, 1989; Reutter and Witt, 1993). In fishes, taste buds occur not only within the oral and pharyngeal cavities, but also on external structures such as the barbels and skin (reviewed by Caprio, 1988; Jakubowski and Whitear, 1990; and others). Excitation of taste buds in the oropharyngeal cavity of two species of catfish, *Ictalurus natalis* and *I. punctatus*, is known to induce swallowing of food, whereas taste buds on the barbels and skin are involved in the location and pickup of food (Atema, 1971; Caprio *et al.*, 1993).

In addition to taste buds, teeth are also present in the oropharyngeal cavities of many fishes. Teeth in the pharynx are frequently associated with pharyngeal jaws, which are situated immediately anterior to the esophagus (Casciotta and Arratia, 1993; Vandewalle *et al.*, 1994, 1995). Pharyngeal jaws and teeth in some species are

involved in the processing of food, whereby it is masticated and crushed before being transported to the esophagus for swallowing (Sibbing, 1982; Claes and De Vree, 1991; Vandewalle *et al.*, 1994, 1995).

Preliminary visual and videotaped studies of feeding in largemouth bass revealed that just before swallowing a goldfish, the bass would always dislodge and expel many scales from the prey (Carr and Netherton, unpub. data). These observations indicated that food processing is occurring in the pharynx, which is also the site of well-developed pharyngeal jaws (Lauder, 1983). An indication that gustation also occurs in the pharynx came from observations of the swallowing or rejection of "food balls" after inhalation. Food balls devoid of a feeding stimulant were spit out after being held in the mouth a few seconds. In contrast, food balls containing a feeding stimulant were swallowed within 30 seconds. Food balls were, incidentally, treated like goldfish: small pieces of food balls were often dislodged and ejected from beneath the operculum—not from the mouth—before the balls were swallowed. This finding not only supports our notion that food processing occurs in the pharynx, it also suggests that prompt interactions occur between gustation and food processing.

Hence in a visual feeder such as the largemouth bass, gustatory signals affecting the final swallowing of suitable prey may be provided by taste buds co-located with the pharyngeal teeth that crush and otherwise damage prey organisms prior to swallowing. Although a 1982 study by Ezeasor in rainbow trout, *Salmo gairdneri*, suggested that the co-location of taste buds and pharyngeal teeth may contribute to both food processing and gustation, this important adaptation is not generally acknowledged. For example, the functional significance of taste buds juxtaposed with pharyngeal teeth near the esophagus is not noted in recent reviews or in other current literature on taste buds, pharyngeal teeth, and gustation in fish (*e.g.*, see Jakubowski and Whitear, 1990; Casciotta and Arratia, 1993; Hara, 1994; Sorensen and Caprio, 1997).

Our initial observations prompted us to study feeding behavior in the largemouth bass in more detail, and to employ light and electron microscopy and cytochemical techniques to determine whether pharyngeal teeth and taste buds occur together in the well-developed pharyngeal jaws. We report here the co-location of large numbers of teeth and taste buds in the pharyngeal jaws situated immediately anterior to the esophagus. Moreover, the results of our behavioral and anatomical studies indicate that this organization of teeth and taste buds functions in food selection in this species.

Materials and Methods

Behavior

Sixteen largemouth bass (*M. salmoides*), 230–305 mm long, were caught by hook and line from a lake in central Florida, and transported to the Whitney Laboratory in an aerated box of lake water. The bass were placed into a 575-liter aquarium of filtered, recirculated well water and fed several times daily with live goldfish, *Carassius auratus* (and occasionally killifish, *Fundulus heteroclitus*). Bricks in the aquarium provided shelters behind which the bass could retreat when alarmed. After 3 weeks of acclimation to captivity, the fish would feed consistently in the presence of an observer, so single fish were next transferred into 76-liter metal-framed glass aquaria. Each aquarium received a constant supply of filtered, aerated well water, and was provided with a brick for shelter. After about 1 month, once the fish had become accustomed to the presence, movements, and siphoning of water and waste material by an observer, the bricks were removed. The sides of the aquaria were covered with an opaque black film to prevent fish from viewing the activities of adjacent animals.

The following regime was then used for about 1 month to condition the test animals to accept "food balls":

1. Bass were fed live goldfish and occasionally killifish at about 5% of body weight per day until at least 12 test animals were eating immediately in the presence of the observer.
2. Feeding with live fish was continued, but the bass were also presented with fresh fish fillets or shrimp pieces threaded onto the end of a monofilament line and moved in front of each test animal. This was continued until all fish were readily accepting the nonliving food.
3. Feeding with live fish was continued. But in addition, diced fish or shrimp was incorporated into "food balls" gelled with carboxymethylcellulose, compressed onto the end of a monofilament line, and presented as described above.
4. Feeding with live fish and food balls was continued, but a shrimp extract or an artificial shrimp mixture replaced the diced flesh in the food balls. Preparation and presentation of the food balls was as above.

Since all largemouth bass were not equally responsive to food on a given day, tests of food balls were preceded by the introduction of a goldfish into the tank of each test animal. On each test day, only bass that inhaled and swallowed the goldfish were subsequently presented with food balls.

Shrimp extract was made by homogenizing defrosted shrimp (*Penaeus sp.*) for 2 min in a blender in cold deionized water (1 g:3 ml; weight:volume). The homogenate was centrifuged at 5°C at 5800 × *g*. The supernatant was removed and referred to as shrimp extract (SE). An artificial shrimp mixture (ASM) was also prepared. The ASM contained the 26 amino acids, quaternary amines, nucleosides, nucleotides, and lactic acid in the same relative concentrations as given in Carr and Derby (1986).

Defined concentrations of SE or ASM were prepared by dilution with deionized water.

Food balls were prepared from a thick malleable gel that was made as follows. One part of sodium carboxymethylcellulose (high viscosity) was mixed with a KitchenAid into 9 parts of liquid composed of defined concentrations of SE, ASM, or deionized water. Portions of the resulting gel, about 2 g each, were shaped by hand into food balls. As a test of their efficacy, food balls were compressed onto the end of a monofilament line and moved in front of individual fish; each fish was given 60 s to respond. The number of food balls inhaled and then either rejected (expelled) or swallowed was recorded. The log likelihood ratio test (Zar, 1984) was used to analyze the data for significant differences.

Some of the tests and other facets of behavior, such as food processing and distinguishing between the roles of vision and gustation, were videotaped with a Sony CCD-V5000 Hi 8 video recorder for further analysis. Chemicals (>98% purity) were obtained from Sigma.

Light microscopy and immunocytochemistry

The pharyngeal jaws together with the gill arches, tongue, and opening to the esophagus were dissected away from sacrificed specimens. The dissected structure was immersed in 4% paraformaldehyde in 0.1 *M* sodium cacodylate buffer, pH 7.2, at 4°C. The tissue was incubated overnight in the primary fixative and then transferred to ice-cold Carnoy's solution (60% ethanol, 30% chloroform, 10% glacial acetic acid) for 90 min. The tissue was next rinsed 3 × 30 min in 100% ethanol followed by clearing in xylene for 3 × 30 min. The gross anatomy of the pharyngeal jaw apparatus was studied and drawn using a Wild M3 dissecting microscope with a camera lucida attachment.

For histological analysis of material prepared as above, specific pharyngeal pads were dissected free of the remaining pharyngeal jaw structure and infiltrated with and embedded in paraffin. Each pharyngeal pad was next sectioned, at 6–15 μm, perpendicular to the longitudinal axis of the pad. Sections were mounted on gel-subbed slides and rehydrated for either immunohistochemical or standard histological staining, as described below.

Some neuronal components of the pharyngeal pads were visualized using indirect immunofluorescence techniques, with the HNK-1 monoclonal antibody (from the American Type Culture Collection) serving as the probe. Secondary antibodies labeled with Texas red and fluorescein (Jackson Research Laboratories, Inc.) were also employed. Further details of tissue structure were also visualized by fluorescent labeling of cell nuclei with 4′,6-diamidino-2-phenylindol (DAPI) as described by Linser *et al.* (1996), and by standard staining with hematoxylin and eosin.

Scanning electron microscopy

The pharyngeal jaws and associated structures described above were dissected away from other parts of the head. The surface of the tissue was rinsed extensively with jets of deionized water to remove some of the mucus and debris. The tissue was fixed in 2.5% glutaraldehyde in 0.1 *M* sodium phosphate buffer (PB), pH 7.4, for 1 to 4 days at 4°C. The tissue was post-fixed in 1% osmium tetroxide in 0.1 *M* PB. The material was then thoroughly rinsed with many volumes of 0.1 *M* PB, and dehydrated in a graded series of ethanol followed by immersion in dimethoxypropane. The tissue was secured onto a stub, vacuum dried, and sputter-coated with gold/palladium (Desk II sputter coater, Denton). The tissue was examined at a voltage of 5–7.5 kV using a Leica S420 scanning electron microscope.

Results

Behavior

Wild largemouth bass, *M. salmoides*, began eating live goldfish after only a few days of acclimation in captivity. Ingestion of live fish consisted of the following steps: inhalation of the prey into the oral cavity, passage through the pharyngeal cavity, and swallowing. Visual observations and video recordings revealed that the swallowing step is always preceded or accompanied by the forceful ejection of many goldfish scales from beneath the operculum. These scales did not appear to be removed during the inhalation process, either by the oral jaws or from within the oral cavity. Indeed, bass were occasionally observed to inhale goldfish, hold them in the oral cavity for several seconds, and then release them unharmed, and with the scales intact. Hence, the scales are apparently removed in the pharyngeal cavity before swallowing.

Captive largemouth bass were readily conditioned to inhale moving food balls. But food balls without a feeding stimulant (= controls) were inhaled as frequently as those containing a natural shrimp extract (SE) or an artificial shrimp mixture (ASM) (Fig. 1 inset). Inhalation of these potential food items was thus induced by the sight of a moving object and not by olfaction or gustation.

Swallowing differs from inhalation in that the decision to swallow a food item occurred only after the object was in the mouth. Food balls flavored with either SE or ASM were swallowed with a frequency that increased in a dose-dependent manner ($P < 0.001$; Fig. 1). Indeed, about 85% of the food balls containing the highest concentration of SE were swallowed, whereas no control food balls were swallowed. Food balls with a concentration of SE greater than 1%, or ASM greater than 0.05%, were swallowed at a significantly greater frequency than the controls ($P < 0.005$). Hence the gustatory sense appears to

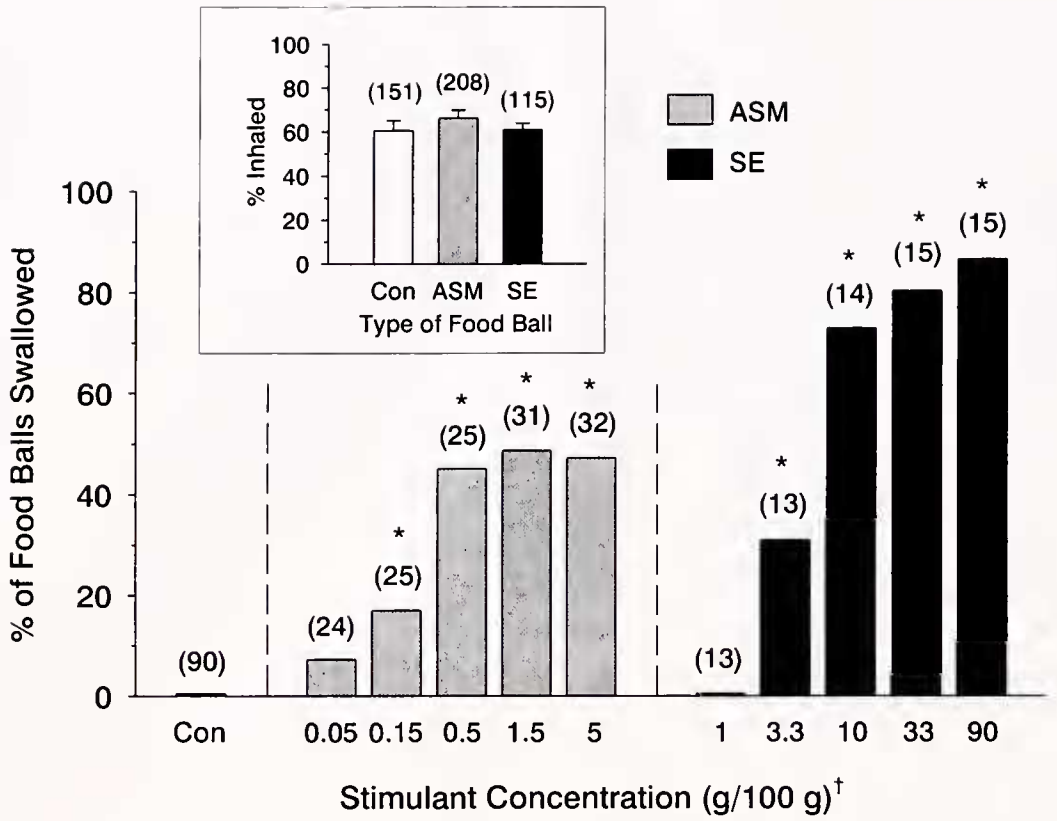

Figure 1. Percentage of food balls with or without feeding stimulant that were inhaled (inset) or swallowed by largemouth bass. Con, control; ASM, artificial shrimp mixture; SE, shrimp extract; parentheses, number of trials. Inhalation of the three types of food balls is not significantly different ($G = 2.38$, df = 2; $P > 0.25$). A significant dose dependency existed for the swallowing of food balls with SE ($G = 36.5$; df = 4; $P < 0.001$) or ASM ($G = 102.6$; df = 4; $P < 0.001$). All food balls with SE > 1.0% and ASM > 0.05% were swallowed with a frequency significantly greater than that of the controls (*; $P < 0.005$). Since inhalation of food balls is a prerequisite to swallowing, the percentage of food balls swallowed was calculated as the number swallowed divided by the number inhaled. †Stimulant concentration: ASM = grams of solute per 100 grams of food ball; SE = grams of SE per 100 grams of food ball.

play a major role in the decision to swallow or reject a food ball, whereas vision is the primary sense affecting its initial inhalation. Once a control ball was in the oral cavity, its average rejection time was about 3 seconds after inhalation (data not given).

Before swallowing a food ball, bass would often dislodge and eject small pieces of the ball from beneath the operculum. This process, like the removal of scales from goldfish, occurred in the posterior part of the oropharyngeal cavity. Control food balls that were rejected after inhalation were usually still intact and were always ejected from the mouth, not the operculum.

Gross morphology of pharyngeal jaws

Figure 2 shows the gross morphology of the pharyngeal jaws, teeth, gill arches, and other associated structures. The upper (dorsal) jaw has three major toothed pads (= pharyngeal pads) on each side of the midline; the most anterior pad is designated as UP1 (Fig. 2A). Each pad has a long and a short axis, with the long axis oriented in the general direction of the esophagus. The most posterior upper pad (UP3) possesses a protuberance that juts into the pharyngeal cavity just anterior to the esophageal entrance. The lower (ventral) pharyngeal pads (LP) consist of single elongate pads on each side of the midline (Fig. 2A). Each LP has a truncated triangular shape, with its posterior margin situated just in front of the entrance to the esophagus. Additional smaller pads of teeth occur on the gill arches and elsewhere in the pharynx (Fig. 2A), but our major focus was upon the major upper and lower pads described above.

All teeth on the pharyngeal pads and elsewhere in the pharynx are caniform. On the upper pads, all teeth are curved, with their tips pointed generally toward the esophagus (Figs. 2B; 3A). The lower pads have straight cani-

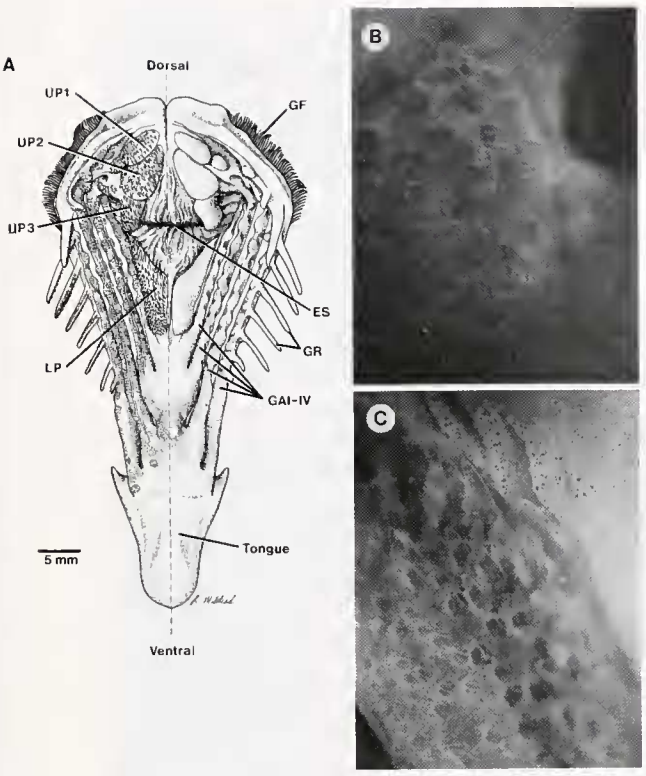

Figure 2. Gross morphology of the pharyngeal jaws, teeth, and associated structures of the largemouth bass. (A) Drawing of the fully distended pharynx and tongue; other parts of the oral cavity are not shown. The structure was opened dorsoventrally to provide a flattened configuration. Details of the dentition on the pharyngeal pads and gill arches are shown on the left half of the figure. Upper (dorsal) pharyngeal pads (UP1–3) and the gill arches (GA I–IV) are numbered in an anterior to posterior direction. ES, esophagus; GF, gill filaments; GR, gill rakers; LP, lower pharyngeal pad. (B) Curved caniform teeth on upper pharyngeal pad, UP2. (C) Straight caniform teeth on medial portion of a lower pharyngeal pad. In panel A, the lines to UP2 and LP terminate in the approximate regions of teeth shown in B and C.

form teeth on the medial half (Fig. 2C) and curved teeth situated toward the periphery, and generally pointed toward the esophagus.

Co-location of pharyngeal teeth and taste buds

Scanning electron microscopy (SEM) showed that taste buds are abundant around and between most teeth of both the upper and lower pharyngeal jaws. Taste buds completely encircle many teeth (Fig. 3B, C). Many taste buds are situated atop distinct mounds or papillae (Fig. 3B–D), whereas others have a flattened surface profile. The relative proportion of the two types of taste buds was not determined because some of the flat buds were difficult to distinguish from other surface structures or debris. Higher magnification SEM showed that each taste bud also had a distinct receptor area, and microplicae (elevated ridges) on the surrounding epithelium (Fig. 3D); these features have been observed in other fish species (*e.g.*, Reutter *et al.*, 1974).

Histological sections verified the existence of taste buds with an elevated surface profile and a flattened profile (Fig. 4); but no further features that might distinguish the function or distribution of the two types were noted. Taste buds were also seen close to teeth on the gill arches (not shown).

Internal structure and immunoreactivity of taste buds on the pharyngeal jaws

Histological sections prepared from all pharyngeal pads showed distinct ovoid taste buds of about 40 μm in diameter and 70 μm in height (Fig. 4A–E). The taste buds

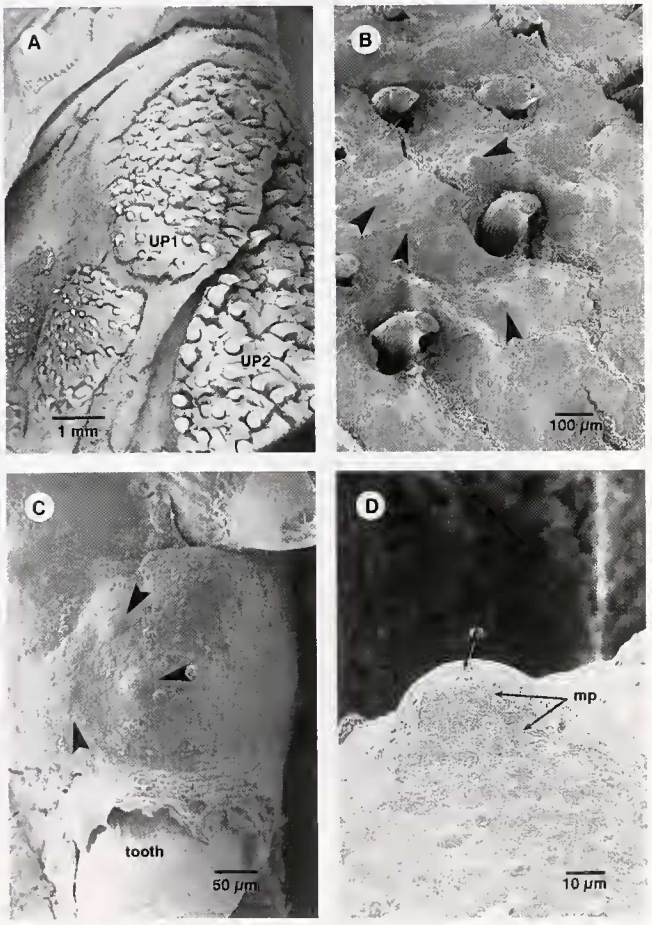

Figure 3. Scanning electron micrographs showing the co-localization of taste buds and pharyngeal teeth on the pharyngeal pads of largemouth bass. (A) Low-magnification view of upper pharyngeal pads, UP1 and UP2, with curved caniform teeth. (B and C) Higher magnification views of papillae of elevated taste buds (arrowheads) co-located with teeth on UP2 (B) and LP (C). (D) Higher magnification of an elevated taste papilla on UP1. mp, microplicae; ra, receptor area. Other abbreviations as in Fig. 2.

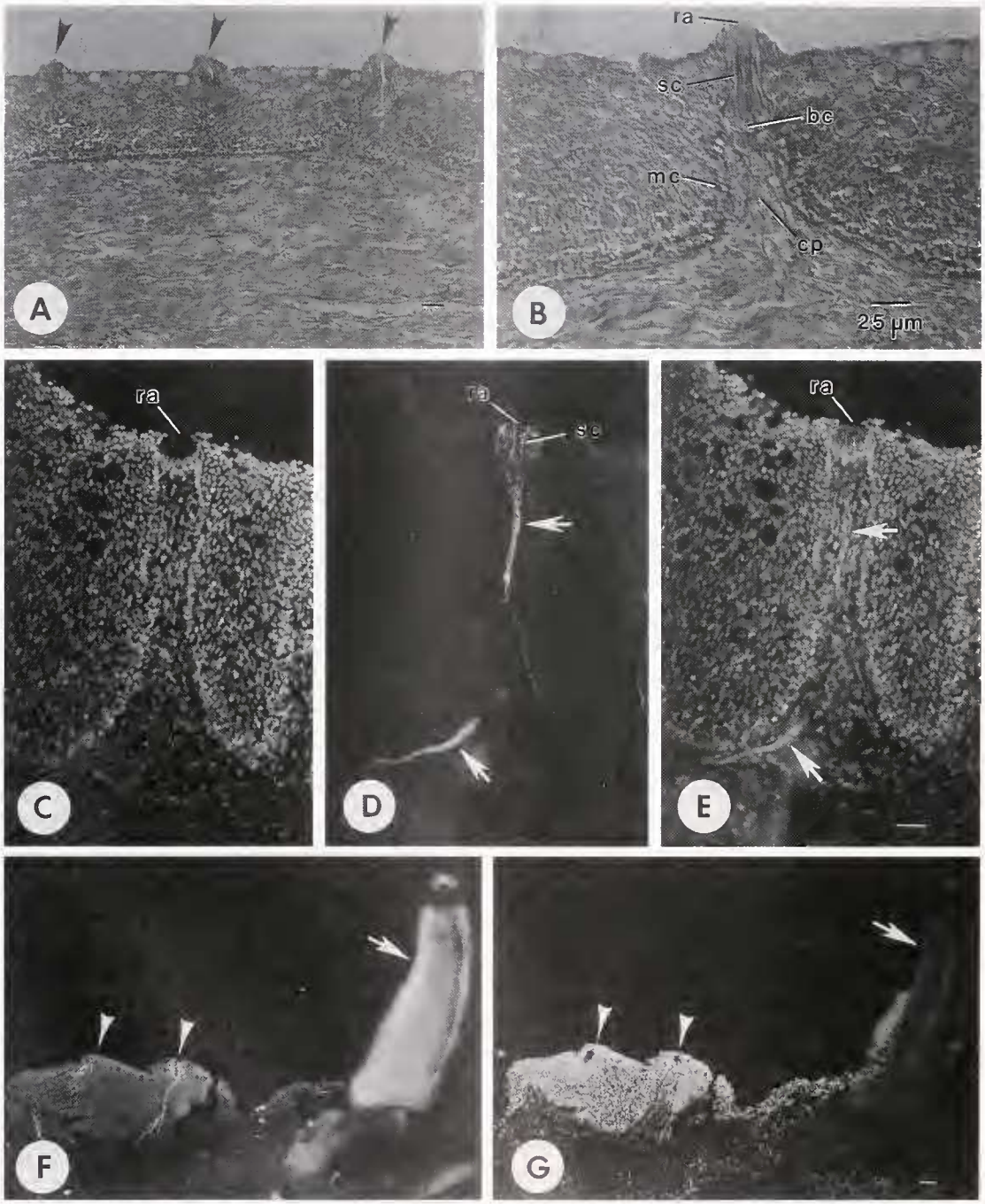

Figure 4. Elevated and flattened taste buds in histological sections of pharyngeal pads of largemouth bass. (A and B) Differential interference contrast images of hematoxylin and eosin stained sections of UP2. (A) Elevated taste buds (arrowheads) in single plane of a section. (B) Higher magnification of an elevated taste bud revealing the details of classical taste bud morphology including sheath of marginal cells (mc), corium papilla (cp), basal cells (bc), receptor area (ra), and elongated putative sensory cells (sc). (C, D, E) Single section from an LP showing a flattened taste bud as revealed by DAPI staining (C), HNK-1 immunofluorescence (D), and a combination of DAPI staining and HNK-1 immunofluorescence (E). HNK-1 fluorescence reveals the relationship between the nerve (arrows) innervating the taste bud and several putative sensory cells (sc) with processes extending into the receptor area (ra) (D and E). Scale bar: 25 μm. (F and G) Single section from UP1 illuminated with HNK-1 immunofluorescence (F) or by DAPI staining (G) at low magnification to show the co-location of a tooth (arrows) and two elevated taste buds (arrowheads). Other abbreviations as in Fig. 2. Scale bar: 25 μm.

appear typical for teleosts (Reutter, 1992; Sorensen and Caprio, 1997). Each exhibits elongated putative sensory cells, basal cells, a corium papilla, associated neuronal elements, and an apical receptor area; and each is enclosed by a sheath of marginal cells (Fig. 4B–D). Other than the differences in surface profile, the general morphology of the elevated and flattened taste buds was very similar.

Aspects of the neural character of taste buds were examined with immunocytochemical techniques. The HNK-1 antibody recognizes a specific carbohydrate moiety frequently found on membrane glycoproteins of neural cells (Linser, 1991; Bakker *et al.*, 1997). In the chick, HNK-1 is a powerful marker of neural crest cells and their derivatives (Bronner-Fraser, 1985). Since the sensory cells of taste buds, as well as other components of the peripheral nervous system, may be of neural crest origin (Gans and Northcutt, 1983; Ganchrow and Ganchrow, 1989), we hypothesized that this antibody might be useful for investigating pharyngeal pads.

Sections of a pharyngeal pad immunostained with the HNK-1 antibody are shown in Figure 4D–F. The staining delimited many nerves coursing through the pads and their supportive tissue. These nerves frequently exhibited branches that turn toward the surface of the pad epithelium, where they enter the basal portion of taste buds. Within a taste bud itself, immunostaining for HNK-1 was also evident over the entire length of one or more of the elongated putative sensory cells, with staining continuing distally directly into the receptor area (Fig. 4D–F). Note that only a few of the sensory cells within each taste bud were labeled with HNK-1 antibody (Fig. 4D, E). Serial analyses of several hundred taste buds revealed that in most cases only one to four cells were immunostained. Additional details of taste bud morphology were obtained by the DAPI staining of cell nuclei (Fig. 4C, E, G). This technique showed that marginal cells clearly delineate the periphery of the taste bud.

Discussion

Food processing is a facet of feeding whereby the condition of a food item is modified by mastication, crushing, or tearing prior to swallowing (Vandewalle *et al.*, 1995). In the current study of largemouth bass, videotaped observations of feeding revealed that after inhaling a live goldfish, bass would remove its scales in the oropharyngeal cavity and eject them from beneath the operculum. These observations strongly suggest that the well-developed pharyngeal jaws of the bass are processing food.

We found that, after largemouth bass inhale gelled food balls, a selection process strongly modulated by gustation occurs within their oropharyngeal cavity. Food balls containing a feeding stimulant were swallowed in a dose-dependent manner, whereas food balls without feeding stimulant were rejected and expelled from the mouth (Fig. 1). Our subsequent finding that large numbers of teeth and taste buds are co-located in the pharyngeal apparatus (Figs. 2–4) indicates that food processing and gustatory sensing occur together in this organ before a potential food item is swallowed.

The co-occurrence of teeth and taste buds in the pharynx of fish has been observed before (Reutter *et al.*, 1974; Ezeasor, 1982; Sibbing, 1982; Hossler and Merchant, 1983; Northcott and Beveridge, 1988). However, these earlier workers failed to include, or to integrate, both behavioral and anatomical evidence demonstrating the interaction of food processing, gustation, and ingestion. For example, in extensive studies of food mastication and transport by the carp, *Cyprinus carpio*, Sibbing (1982) noted the co-occurrence of teeth and taste buds in the pharyngeal apparatus; however, the interaction of food processing and gustation was not mentioned. Likewise, Ezeasor (1982) used scanning and transmission electron microscopy to show that the pharyngeal cavity of the rainbow trout, *Salmo gairdneri*, has both teeth and taste buds, which he concluded could "conceivably" interact to affect the ingestion or rejection of a potential food item. However, Ezeasor did not conduct behavioral studies to show that food processing and gustation do indeed contribute to the decision to swallow.

In the current study with largemouth bass, vision is clearly the major sense inducing inhalation of live fish and food balls, whereas the gustatory sense contributes in a major way to swallowing. Thus these two phases of ingestion are regulated quite differently. In earlier feeding studies with largemouth bass fry, Brandt *et al.* (1987) also showed that food was located by vision, because odorants introduced into the water did not serve as attractants or stimulate feeding behavior. Gustation was, however, important at some point in food ingestion by bass fry because a gustatory stimulant such as freeze-dried krill incorporated into feed pellets increased pellet consumption (Kubitza and Lovshin, 1997). Pellet consumption by largemouth bass was also increased by incorporating artificial mixtures of substances present in a krill extract (Kubitza *et al.*, 1997). The most effective mixtures were those containing the nucleotide and nucleoside, inosine-5′-monophosphate and inosine, plus either eight amino acids or the quaternary amine, betaine. Moreover, in studies with the goldfish, *Carassius auratus*, Lamb and Finger (1995) used gelatin pellets containing feeding stimulants or aversive substances (quinine and caffeine) to show that both gustatory and textural qualities affected the sorting, rejection, or swallowing of pellets after inhalation into the mouth.

The well-developed pharyngeal jaws in largemouth bass and other members of the family Centrarchidae were described by Lauder (1983), who observed that food pro-

cessing in the pharyngeal cavity of centrarchids is especially pronounced in species such as *Lepomis microlophus* and *L. gibbosus*, whose pharyngeal jaws include molariform teeth adapted to crush snails. The adaptation, found in *L. microcephalus*, to discharge shell fragments from crushed snails through the operculum is seemingly similar to the current observations of the discharge of fish scales through the operculum by largemouth bass. However, in contrast to the molariform pharyngeal teeth of *L. microcephalus* and *L. gibbosus*, the largemouth bass has straight and curved caniform teeth (see Figs. 2 and 3) that are more suitable for piercing flesh and removing scales than for crushing very hard objects such as snails. Phylogenetic aspects of the morphology and function of the pharyngeal apparatus in diverse species are described by Liem and Greenwood (1981) and Liem (1986).

The morphology of pharyngeal taste buds in largemouth bass revealed that many are located beneath the apices of elevated epithelial papillae (see Figs. 3 and 4). Hence their gross structure corresponds closely to Type II taste buds described by Reutter *et al.* (1974). Other taste buds were seen with a flattened surface profile that projected only slightly above the surface of the surrounding epithelium, thereby corresponding somewhat to the Type III taste buds described by Reutter *et al.* (1974). Taste buds are not confined to the pharyngeal jaws of the largemouth bass, but also occur throughout the oropharyngeal cavity on the gill arches, palate, tongue, and elsewhere, as described for other species (see reviews by Kapoor *et al.*, 1975; Reutter and Witt, 1993).

In the current study, the mouse antibody HNK-1 stained neurons entering the taste buds, as well as a limited number of putative sensory cells within the taste buds themselves. The HNK-1 antibody recognizes a specific carbohydrate moiety, sulfated glucuronic acid, associated with cell adhesion and cell-cell recognition molecules (Bakker *et al.*, 1997). The HNK-1 antibody has been widely used to examine aspects of neural development because it stains developing and mature neurons, particularly those of neural crest origin (Bronner-Fraser, 1985; Linser, 1991; Linser *et al.*, 1996). Since taste buds may be derived from both neural crest cells and placodal cells of ectodermal origin (Mistretta, 1991), the staining by HNK-1 of only a limited number of sensory cells in taste buds may indicate that these cells are of neural crest origin. Moreover, the distribution of taste bud cells stained with the HNK-1 antibody in the largemouth bass resembles the distribution of taste cells stained with the carbocyanine dye, dil, in the barbels of the catfish, *Ictalurus punctatus* (Finger and Böttger, 1990). In both cases, the limited number of stained cells within taste buds may, to quote the authors of the catfish study, "indicate a special relationship between these cells and the nerve fibers innervating them."

Our behavioral observations of a functional interaction between food processing and gustation provide a logical explanation for the co-localization of taste buds and teeth in the pharyngeal jaws of the largemouth bass. We now propose that in many fish species, food processing and gustation occur together in the pharynx and may serve as final determinants of the suitability of potential food items prior to swallowing. This hypothesis is supported by several findings. Pharyngeal jaws and teeth exist ubiquitously and have a role in food processing and transport in most families of euteleosteans, including members of both the Protacanthopterygii (soft-finned fishes) and Acanthopterygii (ray-finned fishes) (Vandewalle *et al.*, 1994). Furthermore, the presence of both teeth and taste buds within the pharyngeal cavity has been reported for a variety of fish species (Reutter *et al.*, 1974; Ezeasor, 1982; Sibbing, 1982; Hossler and Merchant, 1983; Hossler *et al.*, 1986). Finally, the hypothesis is consistent with our current behavioral and morphological findings with the largemouth bass.

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